Sulphated locust bean gum-coated lipid nanocapsules as potential lung delivery carriers

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Summary

Drugs pertaining to Biopharmaceutics Classification System (BCS) classes II and IV have limitations in their delivery, including in the lung. Therefore, drug delivery carriers have been proposed to improve the therapeutic effectiveness of such drugs. This work proposes lipid nanocapsules (LNC) as a potential platform for lung drug delivery. Locust bean gum (LBG), which is a galactomannan, was used as polymeric shell, protecting the oily core of the nanocapsules and providing their surface with hydrophilic character. Due to the neutral character of LBG, in order to enable nanocapsule formation, a sulphate derivative (LBGS) was prepared, which was confirmed by Fourier-transformed infrared (FTIR) spectroscopy. The electrostatic interaction between the negatively charged sulphate groups of LBGS and the positively charged groups of the used cationic lipid (1,2-dioleoyloxy-3-trimethylammoniumpropanchloride, DOTAP), allowed the formation of monodisperse nanocapsules, with sizes around 200 nm and strongly negative zeta potentials, between -70 and -85 mV. Envisaging potential lung drug delivery, the LBGS-coated LNC were co-formulated with mannitol using spray-drying, producing microencapsulated nanocapsules. Feret's diameter was determined to be 2.6 ± 1.8 µm and 3.1 ± 1.9 µm for Man (control) and Man/LNC microparticles, respectively. Further studies are underway in order to optimise both the nanoplatform and the dry powder formulation.

Key Message

Sulphated LBG-coated lipid nanocapsules are a potential approach on the nanoencapsulation of hydrophobic drugs, which can be microencapsulated to provide inhalable products.

Introduction

Drugs pertaining to Biopharmaceutics Classification System (BCS) classes II and IV experience delivery difficulties. In the last decades, many drug delivery carriers have been proposed to enable their successful application, either by improving the therapeutic efficacy or by decreasing the side effects that many times hamper the clinical use. This reality is transversal to all delivery routes, including the pulmonary. The range of possibilities is wide, including micrometric and nanometric systems that are composed of materials that may have different origins, either natural or synthetic.

The aim of this work was to prepare lipid nanocapsules (LNC) that may be used as carriers of hydrophobic drugs regarding a potential application in lung delivery. Among the various methods described to prepare nanocapsules, solvent displacement is frequently used [1]. Locust bean gum (LBG) was further selected to provide a polymeric coating to the nanocapsules, attributing hydrophilic character to their surface and providing protection to the oily core, where the hydrophobic drugs are located [2]. LBG is a galactomannan extracted from the seeds of *Ceratonia siliqua*, a tree abundant in the Mediterranean region, and especially in the Algarve, the southern region of Portugal [3]. Being devoid of charged groups, the application of LBG in many nanocarrier preparation methods based on electrostatic interactions is hindered, justifying the synthesis of several charged derivatives of the polymer [4, 5]. The interest on LBG relies essentially on its mannose content [6], which provides cell targeting abilities that can be beneficial in the therapy of macrophage-related intracellular diseases or vaccination, for example. Importantly, if lung delivery approaches are envisaged, the nanocapsules need to be endowed with suitable characteristics for inhalation, which can be attributed for instance using spray-drying, freeze-drying or even considering a process of nebulisation. In this work, the production of LNC involved an interaction between a positively charged lipid and a negatively charged derivative of LBG. Moreover, the nanocapsules were further co-spray-dried with mannitol to obtain dry powders that can be applied in inhalation.

Experimental methods

Preparation of LBG sulphate derivative

Commercial LBG (M_w = 589100 Da, Industrial Farense, Portugal) was purified prior to its application. To do so, LBG was dispersed in water, at 85 ºC, and centrifuged (22000 x g, 20 ºC, 1 h) to remove the protein content. The obtained supernatant was precipitated with ethanol and subsequently centrifuged under the same conditions. A SO_3.DMF complex, prepared from chlorosulfonic acid (Merck, Germany) and dry dimethylformamide (VWR, Portugal) was used as sulphating reagent [5]. The success of the reaction was confirmed by Fourier-transform infrared spectroscopy (FTIR, Bruker).

Preparation and characterisation of lipid nanocapsules (LNC)

LNC were prepared by solvent displacement, adapted from [1]. An organic phase comprised of 1,2-dioleoyloxy-3-trimethylammoniumpropanchloride (DOTAP), Miglyol® 812, ethanol and acetone was prepared and poured over the aqueous solution of sulphated LBG (LBGS). The nanocapsules were formed upon contact of both phases, under intense magnetic stirring. Organic solvents were removed by evaporation, reaching a final volume of 10 mL. Different concentrations of DOTAP (0.05% and 0.1%, w/v) and LBGS (from 0.2% to 2%, w/v) were tested to verify their effect on LNC characteristics.

The produced LNC were characterised regarding their size and zeta potential, by diluting the samples in deionised water and in a 0.1 mM KCl solution, respectively (Zetasizer Nano ZS, Malvern Panalytical, UK). The stability of the physicochemical characteristics was evaluated upon storage at 4 ºC, for a period of 3 weeks.

Embedding of LNC

Mannitol (Merck, Germany) was the selected matrix for the embedding of nanocapsules, which was performed by spray-drying. A solution of mannitol (4%, m/v) was prepared in ultrapure water and mixed with the LNC before the spray-drying process. Microparticles of mannitol/LNC = 85/15 (w/w) were prepared using a Buchi B-290 laboratory mini spray-dryer (Buchi Labortechnik AG, Switzerland) equipped with a high-performance cyclone. A spray flow rate of 473 L/h was set, along with inlet temperature of 103 ºC, aspirator at 100% and flow rate at 4.3 mL/min.

The obtained microparticles were characterised regarding Feret's diameter (optical microscopy, VWR) and morphology (scanning electron microscopy - SEM, EVO LS15).

Statistical analysis

A one-way analysis (ANOVA) with the pairwise multiple comparison procedure (Tukey method) was performed to compare multiple groups. All analyses were run using the GraphPad Prism® statistical program (version 6.01), and differences were considered to be significant at a level of $p < 0.05$.

Results and Discussion

The use of LBG in drug delivery approaches has been reported [3, 4], but its neutral character limits the application of many nanocarrier preparation methods that require electrostatic interactions to occur. To circumvent this limitation, the primary step of the work consisted on the synthesis of the sulphate derivative of LBG. Its production was successful, as confirmed by FTIR (Figure 1).

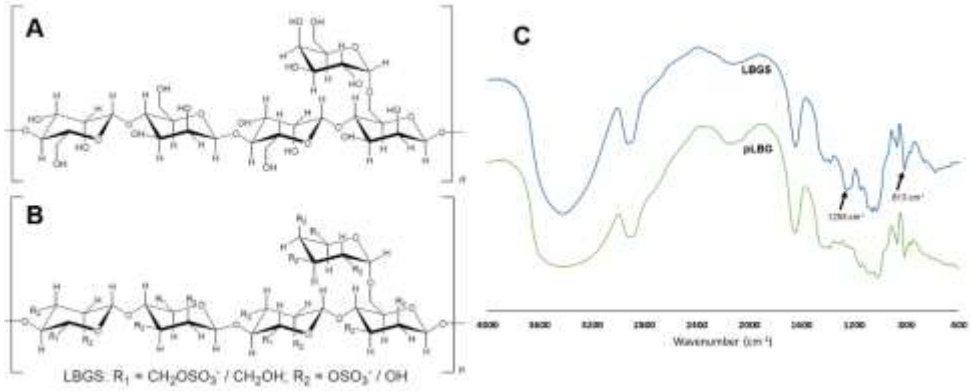

Figure 1 – A. Chemical structure of LBG; B. Chemical structure of the sulphate derivative (LBGS); C. FTIR spectra of purified locust bean gum (pLBG) in green, and its sulphate derivative (LBGS) in blue. Arrows indicate bands characteristic of sulphate groups.

The FTIR spectrum allowed the confirmation of the production of LBGS. Bands at approximately 1255 cm^{-1} and 813 cm^{-1} corresponding to the stretching of S=O and C-O-S bonds, respectively, indicate the presence of sulphate groups.

The LNC were then produced using varied amounts of DOTAP and LBGS and the obtained results are shown in Table 1. The contact between a positively charged oily phase (the core) and a negatively charged aqueous phase (the shell) was promoted. While DOTAP composes the lipid core, LBGS is the external coating, which is corroborated by the negatively charged surface of the nanocapsules.

Table 1 – Physicochemical characteristics of LBGS-coated nanocapsules (mean ± SD).

LBGS/DOTAP (%, w/v)	Size (nm)	PdI	ζ-potential (mV)
0.2/0.05	211 ± 1	< 0.1	-78 ± 2
0.2/0.1	229 ± 1	< 0.2	-72 ± 1
1.0/0.05	193 ± 1	< 0.1	-77 ± 3
1.0/0.1	183 ± 1	< 0.1	-77 ± 1
2.0/0.05	231 ± 5	< 0.1	-78 ± 1
2.0/0.1	194 ± 3	< 0.2	-81 ± 1

The prepared LNC have size around 200 nm with low polydispersity index, indicating monodisperse populations. Varying the amounts of LBG and DOTAP did not have a pronounced effect on nanocapsule characteristics, but the formulation will be further studied, for instance testing intermediate amounts.

The formulation LBGS/DOTAP 0.2/0.05 (%, w/v) was selected to study the evolution of physicochemical characteristics along time and considered representative. Figure 2 shows the variation of size and zeta potential of LNC, upon storage at 4 ºC for 3 weeks.

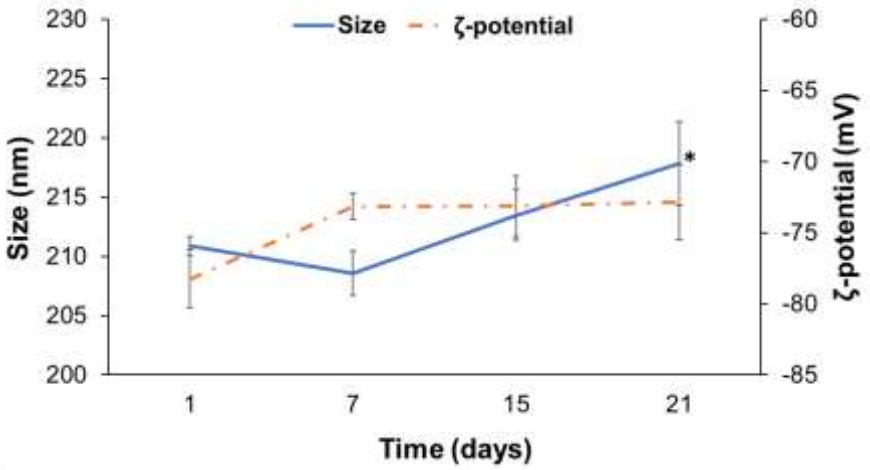

Figure 2 – Evolution of LBGS-coated lipid nanocapsules (LBGS/DOTAP = 0.2%/0.05%, w/v) size (blue) and zeta potential (orange), upon storage at 4 ºC (mean ± SD). * p < 0.05 for the size, compared with day 1.

The zeta potential remained unaltered for the tested timespan. Regarding particle size, although a statistically significant increase was observed between day 1 and 21 ($p < 0.05$), this was only of 7 nm, thus not compromising the objectives of the work.

In order to endow the nanocapsules with suitable characteristics for an application in inhalation, these were embedded using spray-drying. Mannitol (Man) was selected as microparticle matrix material and embedded LNC were produced with a yield of 73%. Both Man and the spray-drying technique were previously demonstrated to be adequate for the embedding of lipid particles [7]. As a preliminary data, Feret's diameter of 2.6 ± 1.8 µm and 3.1 ± 1.9 µm were determined for control microparticles composed only of Man and Man/LNC microparticles, respectively. Figure 3 further shows the morphology of the produced microparticles, which evidence a spherical shape with smooth surface.

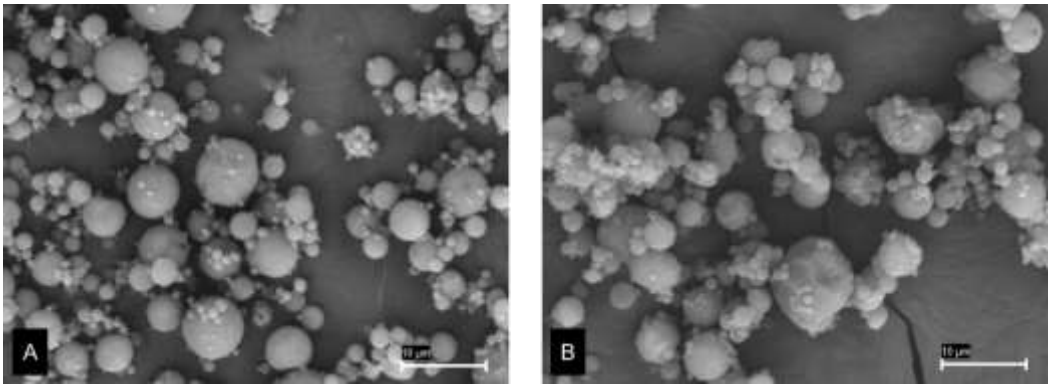

Figure 3 – Representative microphotograph of Mannitol (A) and Mannitol/LNC (85/15, w/w) (B) microparticles obtained by scanning electron microscopy (SEM). Scale bar = 10 µm.

Conclusion

LBG was successfully modified to bear sulphate groups, thus, providing it with a negative charge. Along with DOTAP, this LBG derivative was used to prepare monodisperse LNC with size around 200 nm, independently of the amount of the composing materials. The nanocapsules further evidenced strong negative zeta potential (between -70 and -85 mV), which confirms the presence of LBGS on the surface of the carriers. LNC were successfully embedded in mannitol microparticles produced by spray-drying, providing the needed platform for the application of LNC in lung delivery. Despite the achieved results, both the formulation of nanocapsules and the respective dry powder formulation require further optimisation. The next steps also include the aerodynamic characterisation of the produced powders and the association of a hydrophobic drug.

Acknowledgements

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References

1. Lollo, G., et al., *Enhanced in vivo therapeutic efficacy of plitidepsin-loaded nanocapsules decorated with a new poly-aminoacid-PEG derivative.* International Journal of Pharmaceutics, 2015. **483**(1-2): p. 212-9.
2. Lollo, G., et al., *In vitro anti-cancer activity and pharmacokinetic evaluation of curcumin-loaded lipid nanocapsules.* Materials Science and Engineering C, 2018. **91**: p. 859-867.
3. Alves, A.D., et al., *Inhalable antitubercular therapy mediated by locust bean gum microparticles.* Molecules, 2016. **21**(6): p. 702.
4. Braz, L., et al., *Chitosan/sulfated locust bean gum nanoparticles: In vitro and in vivo evaluation towards an application in oral immunization.* International Journal of Biological Macromolecules, 2017. **96**: p. 786-797.
5. Braz, L., et al., *Synthesis and characterization of locust bean gum derivatives and their application in the production of nanoparticles.* Carbohydrate Polymers, 2018. **181**: p. 974-985.
6. Kawamura, Y., *Carob bean gum - Chemical and Technical Assessment*. 2016, Joint FAO/WHO Expert Committee on Food Additives.
7. Gaspar, D.P., et al., *Microencapsulated solid lipid nanoparticles as a hybrid platform for pulmonary antibiotic delivery.* Molecular Pharmaceutics, 2017. **14**(9): p. 2977-2990.